NON-INTERVENTIONAL STUDY PROTOCOL

TITLE	An Observational Study in Participants with Ryanodine Receptor 1-Related Myopathies (*RYR1*-RM) to Determine Optimal Endpoint Measurements
PROTOCOL/STUDY NO.	CL-EPI-001
VERSION	Version 4.0 10-SEP-2024
SPONSOR	ARMGO Pharma, Inc. 923 Saw Mill River Road, PMB#260 Ardsley, NY 10502, USA
CONDUCTED BY	IQVIA Ltd. The Point, 37 N Wharf Rd London W2 1AF, UK

This protocol contains confidential information that should only be disclosed to those persons responsible for execution and organization of the study and on condition that all such persons agree not to further disseminate it.

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Investigator Signature Page

Study Title: An observational study in participants with Ryanodine Receptor 1-Related Myopathies (*RYR1*-RM) to determine optimal endpoint measurements; Protocol version 1.0 dated 10-NOV-2023

I have read and understand the protocol and agree that it has the ethical, legal, and scientific information necessary to take part in this study. My signature confirms the agreement of both parties that the study will be conducted in accordance with the protocol and all applicable laws and regulations including, but not limited to good pharmacoepidemiology practices, and the ethical principles that have their origins in the Declaration of Helsinki and applicable privacy laws.

I will provide copies of this protocol as needed to all physicians, nurses, and other professional personnel responsible to me who will participate in the study. I will discuss the protocol with them to assure myself they are sufficiently informed about the study's conduct. I am aware that this protocol will need to be approved by an appropriate institutional review board (IRB) or independent ethics committee (IEC) prior to any patients being enrolled and that I am responsible for verifying whether that requirement is met. I agree to adhere to the attached protocol and if asked to provide copies of medical information for verification of submitted information, I will comply.

Since the information in this protocol is confidential, I understand that its disclosure to any third parties, other than those involved in approval, supervision, or conduct of the study is prohibited. I will ensure that the necessary precautions are taken to protect such information from loss, inadvertent disclosure, or access by third parties.

Investigator:

_____

Print Name

_____ _____

Signature Date

_____

Print Name of Institution or Practice and Location

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Reviewed and approved by:

Gene Marcantonio MD PhD		Signed by Gene Marcantonio Gene Marcantonio I approve this document September 12, 2024 | 3:33:31 PM EDT 189079C8EC534F3D8410A3BEC79E3C0B **Signature**		Chief Medical Officer **Title**		September 12, 2024 **Date**
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Elima Jedy-Agba		Signed by Elima Jedy-Agba Elima Jedy-Agba I approve this document September 13, 2024 | 8:08:31 PM JST 450E1A094[illegible]97940F85 **Signature**		Epidemiologist **Title**		September 13, 2024 **Date**
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IQVIA						

Informational Contacts

Sponsor

ARMGO Pharma Inc. will serve as Sponsor of this study. It is the responsibility of the Sponsor to ensure proper monitoring of the study and compliance with all applicable regulatory guidelines and laws.

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Clinical research organization

IQVIA Ltd. will serve as the clinical research organization responsible for the management of the study.

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List of Abbreviations

4SCT	4 Stair Climb Test
10-MWT	10-Meter Walk Test
AE	Adverse event
BMI	Body mass index
CAT	Computerised Adaptive Test
CRF	Case report form
ECG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic data capture
FVC	Forced Vital Capacity
HHD	Hand-held dynamometry
ICF	Informed consent form
IEC	Independent ethics committee
IPAQ	International Physical Activity Questionnaire
IPAQ-SF	International Physical Activity Questionnaire- Short Form
IRB	Institutional review board
MET	Metabolic Equivalent
MMT	Manual Muscle Test
MRC	Medical Research Council
MVIC	Maximum Voluntary Isometric Contraction
PIS	Patient Information Sheet
POC	Proof of concept
PRO	Patient-reported outcome
PROMIS	Patient-Reported Outcomes Measurement Information System

PROMIS-F	Patient-Reported Outcomes Measurement Information System- Fatigue
PROMIS F-SF 7a	Patient-Reported Outcomes Measurement Information System- Short Form v1.0 Fatigue 7a
PROMIS-PF	Patient-Reported Outcomes Measurement Information System-Physical Function
PROMIS PF-SF 10a	Patient-Reported Outcomes Measurement Information System- Short Form v2.0 Physical Function 10a
QMA	Quantitative Muscle Assessment
QMT	Quantitative Muscle Testing
RYR1	Ryanodine receptor isoform 1 gene
RyR1	Ryanodine receptor isoform 1 protein
RYR1-RM	Ryanodine receptor isoform 1-related congenital myopathies
SAE	Serious adverse event
SAP	Statistical analysis plan
SR	Sarcoplasmic reticulum

Study Synopsis

Full Study Title: An Observational Study in Participants with Ryanodine Receptor 1-Related Myopathies (RYR1-RM) to Determine Optimal Endpoint Measurements
Number of Participants: 20 **Duration of Study:** ~9 Months
Background: Ryanodine receptor isoform 1-related myopathies (*RYR1*-RM) are rare, slowly progressive neuromuscular diseases. They are the most common class of congenital myopathies caused by pathogenic variants in the ryanodine receptor isoform 1 *(RYR1*) gene and encompass a heterogeneous spectrum of histopathological and clinical subtypes. The *RYR1*-gene encodes a major skeletal muscle Ca^{2+} release channel - RyR1. RyR1 is embedded within the sarcoplasmic reticulum membrane of skeletal muscle and is a critical component needed for effective skeletal muscle excitation-contraction coupling. Mutations within the *RYR1* gene result in chronic Ca^{2+} leak from the sarcoplasmic reticulum and primarily impair excitation-contraction coupling. Chronic Ca^{2+} leak into the sarcoplasm may lead to increased mitochondrial-related oxidative stress, RyR1 channel oxidation, cellular injury, leading to myopathy. Affected individuals present with mild to severe symptoms ranging from delayed motor milestones, proximal muscle weakness, hypotonia, impaired ambulation, joint contractures, and fatigue to scoliosis, ophthalmoplegia, and respiratory involvement. Although *RYR1*-RM has been associated with significant morbidities and early mortality, there is currently no approved treatment for this debilitating condition. Thus, there is a clear unmet need for therapies to treat *RYR1*-RM. Rycals® are a novel class of Ca^{2+} channel stabilizers that are currently in clinical development. A RyR1 binding site was identified where the Rycal compound, ARM210 (S48168) binds cooperatively with adenosine triphosphate (ATP), stabilizes the closed state of the channel and prevents pathological pore opening. ARM210 (S48168) is expected to be a disease-modifying therapy for *RYR1*-RM patients whose only defect is leaky RyR1. ARM210 (S48168) has completed Phase I clinical studies in healthy volunteers. ARM210 (S48168) is safe and well tolerated in single and multiple dose studies and has now been tested in 7 patients with *RYR1*-RM. Before progressing to Phase II there is a need to define appropriate endpoints. This non-interventional study plans to provide evidence to support the optimal endpoints for a Phase II study.
Rationale: This study aims to assess the extent to which the strength of proximal muscle movements is affected in patients with *RYR1*-RM with autosomal dominant mutations and the number of measurements for these movements to establish a stable baseline of the strength in these patients. These data will also be complemented and evaluated with added evidence from patient-reported outcome (PRO) measures. Muscle strength assessments in this study will be performed using the Quantitative Muscle Assessment (QMA), Manual Muscle Testing (MMT), and Hand-Held Dynamometry (HHD). The 10 Meter Walk Test (10-MWT), 1-Minute Sit-to-Stand Test, and 4 Stair Climb Test (4SCT) are additional tests to be conducted in this study to assess muscle strength and functionality. The endpoints selected for QMA are all proximal muscle movements that are often affected in *RYR1*-RM patients. The study also seeks to describe PROs using the Patient-Reported Outcomes Measurement Information System (PROMIS)-Fatigue, PROMIS-Physical Function (PROMIS-PF), International Physical Activity Questionnaire (IPAQ), and symptom diary. The Syde® device will also be used in this study to objectively assess real world activity (e.g., stride velocity when walking and climbing stairs) in ambulatory patients with *RYR1*-RM.
Objectives: Primary objective • To determine the extent to which muscle strength is affected in participants with *RYR1*-RM with autosomal dominant mutations and describe measurements over time. Secondary objectives • To determine the extent to which fatigue and physical function is affected in participants with *RYR1*-RM with autosomal dominant mutations. • To describe the demographic and clinical characteristics of participants with *RYR1*-RM with autosomal dominant mutations in the real-world setting. Exploratory objective • To evaluate the Syde® device for use in participants with *RYR1*-RM with autosomal dominant mutations.
Study Design: This is an observational, prospective, multi-centre study to assess muscle strength in participants with *RYR1*-RM with autosomal dominant mutations. The study will consist of up to 4 visits, each taking place approximately 30 days apart with the following windows of deviation to allow for flexibility in participant scheduling defined as follows: Screening Visit

(0 days), Visit 1 (±3 days), Visit 2 (-14/ +3 days) and End of Study Visit (-14/ +3 days). Therefore, the length of time for a participant to complete all four visits would range from a minimum of 59 days to a maximum of 99 days. The overall duration of the study will be up to 9 months, from first data collection to reporting of results.

Study Population: The study will aim to enrol up to 20 participants from neurology clinics at study centres in Europe and the United Kingdom over a 2.5-month enrolment period.

Inclusion Criteria

The following criteria must be met to be enrolled in the study:

- Male and female patients (biological sex*) aged 18 years or older at Screening;
- Confirmed genetic diagnosis of *RYR1*-RM with autosomal dominant mutation and supporting clinical phenotype with demonstrable proximal weakness on at least one of the baseline study assessments;
- Evidence of at least one demonstratable muscle/motor function deficit assessed through MMT and scored using the MRC Scale for muscle strength on physical examination;
- Able to walk 10 meters, with or without assistance - e.g., with a cane (assessed using the 10-MWT);
- Willingness and ability to comply with scheduled visits, and study procedures;
- Willingness to be fitted with the Syde® device at Screening Visit (for inclusion in the exploratory objective only); and
- Able to provide written informed consent and understand the study procedures in the informed consent form (ICF).

Exclusion Criteria

Patients meeting at least one of the following criteria will not be eligible for the study:

- Severe pulmonary dysfunction at Screening (FVC < 40% predicted) or evidence of pulmonary exacerbation (note that pulmonary exacerbations refer to acute worsening respiratory symptoms resulting from a decline in lung function);
- Significant cognitive impairment in the judgement of the investigator who will be unable to follow the protocol;
- Patients with progressive neurological conditions (e.g., Parkinson's disease);
- Non-ambulant patients; or
- Pregnant women.

*Biological sex of male or female is required for study inclusion based on the available normative values for the tests conducted, but all gender identities are eligible for inclusion in the study.

Data Collection/Data Sources: Data will be collected during visits to the site and extracted from participant medical records (paper and/or electronic). The following data will be collected for all enrolled participants at:

<u>Screening Visit</u>

- Informed consent;
- Inclusion/Exclusion (including MMT to assess muscle/motor function deficit, 10-MWT to assess ability to walk, and FVC to assess pulmonary dysfunction);
- Demographics (medical records);
- Medical history (medical records) ;
- Medication use (medical records);
- Vital signs and ECG (medical records or primary data collection; note that FVC will be evaluated as part of the assessment of the exclusion criteria);
- Strength measurements using QMA and HHD (note that MMT will be conducted at the start of the Screening Visit as part of the assessment of the inclusion criteria);
- 1-Minute Sit-to-Stand Test;
- 4SCT;
- Full physical, neurological, and functional examination;
- PROMIS Questionnaires;
- IPAQ;

- Syde® device fitting;
- Symptom diary (initiation); and
- Adverse events (AE)/Serious adverse events (SAE).

Visit 1, Visit 2 and End of Study Visit

- Medication use;
- Strength measurements using QMA, HHD, and MMT;
- 10-MWT;
- 1-Minute Sit-to-Stand Test;
- 4SCT;
- Syde® device removal (at Visit 1 only);
- Symptom Diary (to be completed daily between Screening Visit and End of Study Visit); and
- AE/SAE .

End of Study Visit

- PROMIS Questionnaires;
- IPAQ;
- Full physical, neurological, and functional examination

Data Management and Quality Assurance: A data management plan will be created before data collection begins and will describe all functions, processes, and specifications for data collection, cleaning, and validation. The electronic case report forms (eCRFs) will include programmable edits to obtain immediate feedback if data are missing, out of range, illogical or potentially erroneous. A concurrent manual data review will be performed based on parameters dictated by the plan. Ad hoc queries will be generated within the EDC system and followed up for resolution. High data quality standards will be kept, and processes and procedures will be used to repeatedly ensure that the data are as clean and correct as possible when presented for analysis. Data quality will be enhanced through a series of programmed data quality checks that automatically detect out of range or anomalous data.

A study quality assurance and monitoring plan, which is appropriate for the study design, will be developed and implemented. During the remote site initiation visit, the monitor will provide training on the conduct of the study to the investigator, co-investigator(s), and all site staff involved in the study. To ensure the integrity of the data, sites will be monitored. Remote site monitoring will be performed by IQVIA clinical research associates (CRAs) or Sponsor representatives to examine compliance with the protocol and adherence to the data collection procedures, to assess the accuracy and completeness of submitted clinical data and to verify that records and documents are being properly maintained for the duration of the study. The monitor will perform source data verification by reviewing the original patient records. The monitor will close out each site remotely after the last participant's final follow-up assessment is completed, all data have been entered and all outstanding monitoring issues have been resolved or addressed. All monitoring procedures and the frequency of monitoring visits will be described in a monitoring plan. Monitor contact details for each participating site will be maintained in the Investigator Site File. Representatives of the Sponsor's quality assurance unit/monitoring team and competent regulatory authorities must be permitted to inspect all study-related documents and other materials at the site, including the Investigator Site File, the completed eCRFs and the patients' original medical records. Audits may be conducted at any time during or after the study to ensure the validity and integrity of the study data.

Safety: All AEs, regardless of their relationship to use of the Syde® medical device or study procedures will be monitored and reported throughout the study. The AE reporting period begins when the participant is included into the study (date of first signature of informed consent) and continues till the end of study as defined by the Sponsor. AEs will be recorded on the appropriate forms (e.g., case report forms [CRFs], eCRFs) as designated by the Sponsor. In addition to recording the event on the CRF, all SAEs and non-serious AEs considered related to the Syde® medical device or study procedures, reported during study visits may also need to be reported to local health authorities and regional bodies as per local guidelines. The participating physician is responsible for maintaining compliance with local guidelines as well as with any applicable site-specific requirements related to the reporting of SAEs or other safety information to the local institutional review board/independent ethics committee (IRB/IEC) that approved the study.

Statistical Considerations: All computations and generation of tables, listings, and data for figures will be performed using statistical analysis software (SAS®) version 9.2 or higher (SAS Institute, Cary, NC, USA). Prior to the database lock, a detailed, finalized statistical analysis plan (SAP) will be completed and approved. The SAP will contain a more comprehensive description of the methodology for the statistical analyses than outlined in this protocol. The SAP will provide full details of the analyses, the data displays, and the algorithms to be used for data derivations.

Continuous variables will be described using descriptive statistics of central tendency (median and mean) and dispersion (standard deviation, interquartile range, maximum and minimum values). Categorical variables will be summarized as counts and proportions of the total study population. The 95% confidence interval will be presented for estimates of means and proportions. Descriptive statistics for both continuous and categorical variables will be based on participants with non-missing responses to the variable. Frequencies and percentages of missing values in each variable will be reported.

Whenever applicable, the endpoints will be summarized by each visit and across the overall study period (e.g., average endpoint across study visits). Furthermore, absolute changes in the endpoints between (i) Screening and each follow-up visit, and (ii) between each consecutive follow-up visit will be calculated and summarized with descriptive statistics.

Primary objective analysis

Muscle strength endpoints measured through the QMA, HHD, MMT, 10-MWT, 1-Minute Sit-to-Stand Test, and 4SCTwill be summarized using descriptive statistics, as detailed above (including absolute values and changes). The variability of the primary study endpoints between repeated measurements and/or visits will be assessed using graphical methods and correlation coefficients. Further details about the statistical methodology, including any comparison to available normative data will be included in the SAP.

Secondary objective analysis

The PROMIS Fatigue, PROMIS-PF, and IPAQ will be scored according to instructions in their manual. PRO endpoints assessed through the PROMIS Fatigue scale, PROMIS-PF, and IPAQ, and symptom diary will be summarized using appropriate descriptive statistics, as outlined above.

Demographic and clinical characteristics of participants will be summarized with descriptive statistics, as outlined above. AEs recorded throughout the study will also be summarized through descriptive analysis.

Exploratory objective analysis

Explorative endpoints will be summarized using descriptive statistics, as appropriate.

Additional exploratory analyses

Additional exploratory analyses may be performed and outlined in the SAP before the analysis.

Sample Size

The study plans to enrol up to 20 participants. This sample size is based on the number of participants that will be feasible to recruit at the participating sites. Given the explorative and descriptive nature of this study and the rarity of the target population, formal sample size calculations were not performed. Accordingly, the primary focus of the study is on measuring and describing the study endpoints in the target population rather than on statistical hypothesis testing.

Interim and Final Analysis

As this is a non-interventional observational study the data will be assessed on an ongoing basis for accuracy and completeness, however full reporting will only be done when all participants have completed, and the study is considered closed.

A final study report will be generated after all data collection is complete. The final report will encompass all planned analyses, including a description of the complete study population, as described above and in the SAP.

Ethical and Regulatory Considerations: To ensure the quality and integrity of research, this study will be conducted under the guidelines for good pharmacoepidemiology practices issued by the International Society for Pharmacoepidemiology, the Declaration of Helsinki and its amendments, and any applicable national guidelines**.** Prior to the enrolment of any participants in the study, the following documents must be provided by the site to the Sponsor (or their designee):

- Copy of the IRB/IEC approval letter for the protocol and informed consent (all written information provided to the participant must be approved by the IRB/IEC)
- Copy of the IRB/IEC approved informed consent document to be used
- Copy of the protocol signature page signed by the investigator
- Fully executed site agreement

An ICF must be signed by the participant before his or her participation in the study. The medical file for each participant should document the informed consent process and that written informed consent was obtained prior to participation in the

study. A copy of each signed ICF must be provided to the participant. If applicable, it will be provided in a certified translation of the local language. All signed and dated ICFs must remain in each participant's study file and must be available for verification by study monitors at any time. The ICF should be revised whenever there are changes to procedures outlined in the informed consent or when added information becomes available that may affect the willingness of the participant to participate. For any updated or revised ICFs, the medical file for each participant should document the informed consent process and that written informed consent was obtained for the updated/revised ICF for continued participation in the study.

To maintain participant confidentiality, each participant will be assigned a unique participant identifier upon study enrolment. This participant identifier will be used in place of participant name for data analysis and reporting. Medical record numbers or other local reference identifiers are not collected as part of the database. All parties will ensure the protection of participant personal data and will not include participant names on any study forms, reports, publications, or in any other disclosures, except where required by law. In accordance with local regulations in each of the registry countries, participants will be informed about data handling procedures and asked for their consent. Data protection and privacy regulations will be observed in capturing, forwarding, processing, and storing participant data. Every effort will be made to protect participant confidentiality in compliance with the Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons about the processing of personal data and on the free movement of such data and repealing Directive 95/46/EC (General Data Protection Regulation).

The database will be housed at IQVIA in a physically and logically secure computer system kept by IQVIA as per a written security policy. The system meets approved, established standards for the security of health information and is validated. The system also meets the standards of the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use Good Clinical Practice E6 guideline (revision 2) regarding electronic study data handling and is available for audit upon request.

Consistent with local regulations and prior to enrolment of participants at a given site, the study protocol will be submitted together with its associated documents (e.g., ICF, information sheet) to the IRB/IEC responsible for its review. Participant enrolment will not start at any site before the Sponsor has obtained written confirmation of a favourable opinion/approval from the relevant central or local IRB/IEC. The IRB/IEC will be asked to provide documentation of the date of the meeting at which the favourable opinion/approval was given that clearly identifies the study, the protocol version, and the ICF version reviewed. Before implementation of any substantial changes to the protocol, protocol amendments will also be submitted to the relevant IRB/IEC in a manner consistent with local regulations. Pertinent safety information will be submitted to the relevant IECs during the study in accordance with local regulations and requirements. It is the responsibility of the investigator to have prospective approval of the study protocol, protocol amendments, and ICF, and other relevant documents, if applicable, from their local IRB/IEC and provide documentation of approval to the Sponsor or IQVIA. All correspondence with the IRB/IEC should be retained in the investigator file. Should the study be terminated early for any unanticipated reason, the investigator will be responsible for informing the IRB/IEC of the early termination.

Documentation of Protocol Amendments

Date	Section of study protocol	Amendment or update	Reason
December 2023	Exclusion criteria (Section 4.2.2)	Version 2.0	Exclusion criteria have been amended to also exclude pregnant women.
December 2023	Primary Endpoint Measures (Section 4.4.1.1)	Version 2.0	Description of primary endpoint measures has been amended to include more detailed information about the procedures, scoring, and reference values of the tests.
December 2023	Secondary Endpoint Measures (Section 4.4.1.2)	Version 2.0	*a) PROMIS Fatigue, PROMIS-PF, and IPAQ questionnaires:* Description of questionnaires has been amended to include more information about their scoring and clinical value. *b) Demographics and clinical characteristics:* List of tests performed as part of the physical, neurological, and functional examination has been updated to only include tests that are relevant to the scope of the study and explain how they will be scored. *c) Diary:* It has been clarified that participants will record symptoms into the diary on a continuous basis, starting from the Screening Visit until the End of Study Visit.
December 2023	Data Sources and Collection (4.5)	Version 2.0	The expected duration of the visits has been included. The tests have been listed in the order in which they will be administered. It has been clarified that Vital signs, FVC and ECG will be assessed at the time of the Screening Visit or based on the most recent assessment captured in the participants' medical records within 12 months prior to the Screening Visit.

February 2024	Study Synopsis Inclusion criteria (4.2.1)	Version 3.0	The inclusion criteria have been amended to include all male and female participants (biological sex*) aged 18 years and older at Screening. A comment has also been included below the inclusion/exclusion criteria stating that biological sex of male or female is required for study inclusion based on the available normative values for the tests conducted, but all gender identities are eligible for inclusion in the study.
February 2024	End of Study Definition (4.5.4)	Version 3.0	The End of Study is defined as the date of the last query resolution (i.e. all data has been recorded in the electronic case report form and all data queries resolved to allow database lock to occur. This will ensure that all data is available to answer the research questions in the study protocol. This definition will be used across all participating sites.
August/September 2024	Study Synopsis Study Description (4.1) Data Sources (4.5) Multiple sections (“patient” to participant”)	Version 4.0	The study visit schedule has been amended to clarify that there will be up to 4 study visits, and each visit is scheduled to take place 30 days apart, with a window of flexibility in participant scheduling defined as follows: Screening Visit (0 days), Visit 1 (±3 days), Visit 2 (-14/ +3 days) and End of Study Visit (-14/ +3 days). Therefore, the length of time for a participant to complete all four visits would range from a minimum of 59 days to a maximum of 99 days. The overall duration of the study will be up to 9 months, from first data collection to reporting of results. “Patient” has been updated to “participant” where the sentence refers to a consented patient in the study.

1. BACKGROUND

Ryanodine receptor isoform 1-related myopathies (*RYR1*-RM) are rare, slowly progressive neuromuscular diseases. (1) They are the most common class of congenital myopathies caused by pathogenic variants in the ryanodine receptor isoform 1 *(RYR1*) gene and encompass a heterogeneous spectrum of histopathological and clinical subtypes. (1)

The *RYR1*-gene encodes a major skeletal muscle calcium (Ca^{2+}) release channel – RyR1. RyR1 is embedded within the sarcoplasmic reticulum (SR) membrane of skeletal muscle and is a critical component required for effective skeletal muscle excitation-contraction coupling. (1, 2) Mutations within the *RYR1* gene result in chronic Ca^{2+} leak from the SR and primarily impair excitation-contraction coupling. (3, 4) Chronic Ca^{2+} leak into the sarcoplasm may lead to increased mitochondrial-related oxidative stress, RyR1 channel oxidation, cellular injury, leading to myopathy. (1, 5) Patients affected by *RYR1*-RM may experience mild to severe symptoms, ranging from delayed motor milestones, proximal muscle weakness, hypotonia, impaired ambulation, joint contractures, and fatigue to scoliosis, ophthalmoplegia, and respiratory involvement. (2, 3)

Both autosomal dominant (monoallelic, including *de novo* pathogenic variants) and recessive (biallelic) patterns of inheritance are reported to cause *RYR1*-RM. Although *RYR1*-RM has been associated with significant morbidities and early mortality, there is currently no approved treatment for this debilitating condition. Thus, there is a clear unmet need for therapies to treat *RYR1*-RM.

Rycals® are a novel class of Ca^{2+} channel stabilizers that are currently in clinical development. Under normal physiological conditions, Calstabin1 (FKBP12) binds and stabilizes the RyR1 closed state. (5) However, preclinical studies suggest that RyR1-Calstabin1 association is decreased in *RYR1*-RM, which exacerbates SR Ca^{2+} leak, resulting in detrimental downstream effects on muscle function. Rycals function as Ca^{2+} channel stabilizers by restoring RyR1-Calstabin1 binding when it is deficient, thereby stabilizing the RyR1 closed state. (5, 6)

The structural basis for mutations in RyR1 and the mechanism of action of ARM210 (S48168) on mutant forms of RyR1 has recently been elucidated. Using a series of cryo-electron microscopy (cryoEM) structures, Melville et al. (2022) identified a RyR1 binding site where the Rycal compound, ARM210 (S48168) binds cooperatively with adenosine triphosphate (ATP), stabilizes the closed state of the channel and prevents pathological pore opening. (7) ARM210 (S48168) is expected to be a disease-modifying therapy for *RYR1*-RM patients whose only defect is leaky RyR1.

ARM210 (S48168) has completed Phase I clinical studies in healthy volunteers. ARM210 (S48168) is safe and well tolerated in single and multiple dose studies and has now been tested in 7 patients with *RYR1*-RM (NCT04141670). (8) Before progressing to Phase II, there is a need to define appropriate endpoints. This non-interventional study plans to provide evidence to support the optimal endpoints for a Phase II study in *RYR1*-RM patients.

2. RATIONALE

Patients with *RYR1*-RM often have weakness in proximal muscle (closer to the trunk) movements rather than distal movements. (2) Improvements in shoulder abduction that involves proximal muscle have been observed in a Phase 1 clinical study with ARM210 (S48168). (9) This study aims to assess the extent to which the strength of movements is affected in participants with *RYR1*-RM with autosomal dominant mutations and the number of measurements for these movements to establish a stable baseline of the strength in these participants. These data will also be complemented and evaluated with added evidence from patient-reported outcome (PRO) measures. The study results will be used to inform anticipated variability and baseline measurements on the planned endpoints for a Phase II study. The findings from observed variability in muscle strength endpoints from this study will be compared to reference values obtained from the literature for healthy individuals. Muscle strength assessments in this study will be performed using Quantitative Muscle Assessment (QMA), Manual Muscle Testing (MMT), and Hand-Held Dynamometry (HHD). QMA is a computer-based system that tests muscle strength and fatigue by measuring the Maximum Voluntary Isometric Contraction (MVIC) of specific muscle movements. The participant pulls or pushes against a strap that is connected to an adjustable frame. The force measurements are compiled using a fixed dynamometer. This is the most quantitative method for measuring strength and will be used to determine the optimal endpoints to take into future studies. The endpoints selected for QMA are all proximal muscle movements that are often affected in *RYR1*-RM patients. In this study, endpoint measurements will be performed multiple times to determine how many separate measurements will allow for a stable baseline and to assess variability. It is expected that, with learning or education effect, participants may improve their performance on repeated measurements of the QMA endpoints as they become more familiar with the apparatus or procedure, without improving their strength per se. MMT, which is scored using a 0-5 point Medical Research Council (MRC) muscle strength scale, and HHD, an hand-held device which also measures the MVIC generated from a muscle group, are two common methods to assess muscle strength in patients with myopathies in previous studies (10) and will be used in this study. The10 Meter Walk Test (10-MWT), 1-Minute Sit-to-Stand Test, and Stair Climb Test are the additional tests to be conducted in this study to assess muscle strength and functionality. The 10MWT has been used to assess performance of patients with Duchenne Muscular Dystrophy (11) and the 1-Minute Sit-to-Stand Test has been used to assess both

functional exercise capacity and quadriceps strength. (12, 13) The Stair Climb Test assesses the ability to climb a flight of stairs and lower extremity strength, power, and balance. (14, 15)

The study also seeks to describe PROs using the Patient-Reported Outcomes Measurement Information System (PROMIS)-Fatigue, PROMIS-Physical Function (PROMIS-PF), International Physical Activity Questionnaire (IPAQ), and symptom diary. Fatigue is one of the most predominant features of chronic illness. In the health outcomes measurement perspective, fatigue is defined as an overwhelming, debilitating, and sustained sense of exhaustion that reduces an individual's ability to carry out daily activities, including the ability to work effectively or function at optimally. (16) It is expected that information on the subjective experience of the participant collected using these PROs would complement the objective assessments conducted using QMA and other muscle strength tests. The PROMIS Fatigue scale will be used as a measure of fatigue severity in this study. Improvements in the PROMIS Fatigue scores for autosomal dominant patients were observed in the Phase 1 trial that was recently completed in *RYR1*-RM patients. Furthermore, the PROMIS Physical Function (PROMIS-PF) will be used as a measure of physical functioning, upper-extremity function, and mobility. *RYR1*-RM participants will also use a diary to capture their symptoms. Lastly, the Syde® device will be used in this study to objectively assess activity levels in ambulatory participants with *RYR1*-RM.

3. OBJECTIVES AND ENDPOINTS

Table 1. Study objectives and endpoints

Objectives	Endpoints
Primary	
• To determine the extent to which muscle strength is affected in participants with *RYR1*-RM with autosomal dominant mutations and describe measurements over time.	• Weight-scaled muscle strength as measured by QMA (measuring participant's shoulder abduction, elbow flexion and extension, and knee flexion and extension), MMT (measuring participant's neck flexion), and HHD (measuring participant's neck flexion) • Muscle strength will also be measured by additional tests:10-MWT, 1-Minute-Sit-to-Stand Test, and Stair Climb Test.
Secondary	
• To determine the extent to which fatigue and physical function is affected in participants with *RYR1*-RM with autosomal dominant mutations.	• PROMIS Fatigue, PROMIS-PF and, IPAQ questionnaire outputs.
• To describe the demographic and clinical characteristics of participants with *RYR1*-RM with autosomal dominant mutations in the real-world setting.	• Demographics and clinical characteristics (medication use including current and prior medications, medical history, full physical, neurological, and functional examination, height, weight, BMI, and vital signs). • Symptom diary.
Exploratory	
• To evaluate the Syde® device for use in participants with *RYR1*-RM with autosomal dominant mutations.	• Syde® device use in participants with *RYR1*-RM through objective assessment of activity (e.g., stride velocity when walking and climbing stairs).

10-MWT, 10- Meter Walk Test; BMI, Body mass index; IPAQ, International Physical Activity questionnaire; PROs, patient-reported outcomes; PROMIS, Patient-Reported Outcomes Measurement Information System; PROMIS-PF, Patient-Reported Outcomes Measurement Information System-Physical Function; *RYR1*-RM, Ryanodine receptor isoform 1-related congenital myopathies; QMA, Quantitative Muscle Assessment.

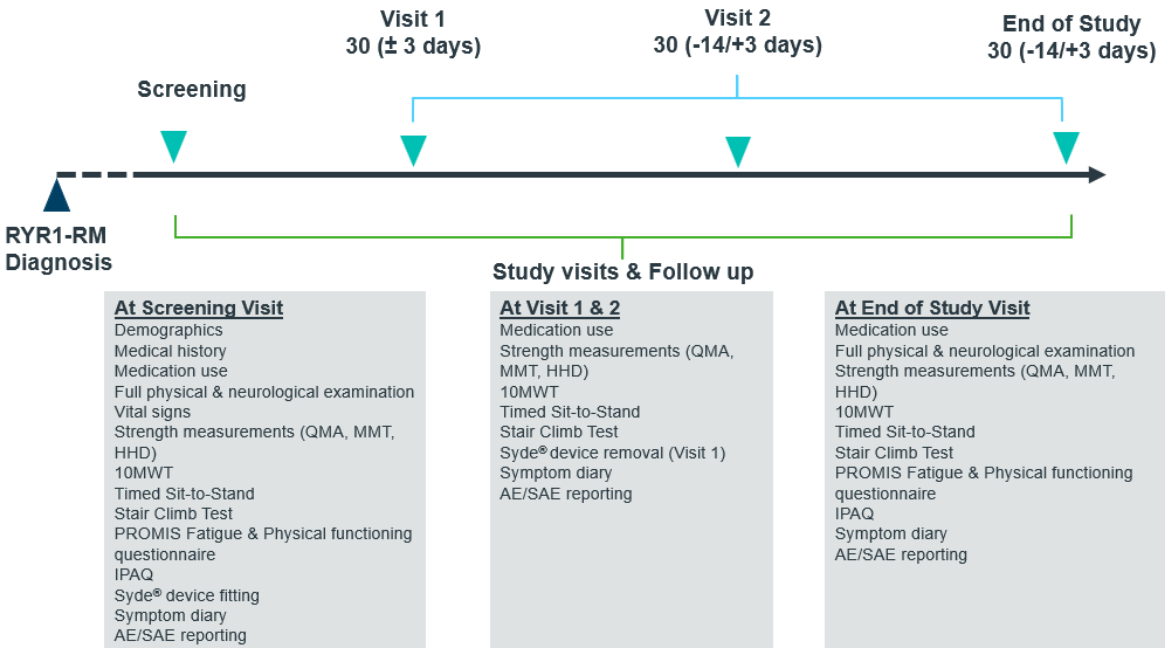
4. STUDY DESIGN

4.1 Study Description

This is an observational, prospective, multi-centre study to assess muscle strength in participants with *RYR1*-RM with autosomal dominant mutations to determine the optimal muscle strength measurement endpoints. The study will be conducted at participating sites in Europe and the United Kingdom.

The study will consist of up to four visits: each taking place 30 days apart with the following windows of deviation to allow for flexibility in participant scheduling defined as follows: Screening Visit (0 days), Visit 1 (±3 days), Visit 2 (-14/ +3 days) and End of Study Visit (-14/ +3 days). The length of time for a patient to complete all four visits would range from a minimum of 59 days to a maximum of 99 days. Index date is defined as the date of the participant's Screening Visit. Six muscle movements dependent primarily on the proximal muscles will be tested to inform optimal endpoints for further studies. At each study visit including Screening, participants' shoulder abduction, elbow flexion and extension, and knee extension and flexion will be measured using QMA testing. Neck flexion will be measured using HHD and MMT. The study's overall duration will be about 9 months, from data collection to analysis and reporting of results. A schematic of the study design is presented in Figure 1.

Figure 1 Study Design



10-MWT, 10-Meter Walk Test, AE, adverse event; HHD, Hand-held dynamometry; IPAQ, International Physical Activity questionnaire; MMT, Manual Muscle Test; PROMIS, Patient-Reported Outcomes Measurement Information; *RYR1* RM, Ryanodine receptor isoform 1-related congenital myopathies, SAE, serious adverse event; QMA, Quantitative Muscle Assessment.

Data will be extracted from the participants' medical records (both paper and/or electronic) at the Screening Visit (at site) and entered in an electronic case report form (eCRF). This data will be used to capture the participant's full medical history (starting from the earliest record available), including the date of *RYR1*-RM diagnosis, comorbidities, and medication use. Medical records may also be used to assess other clinical characteristics, including vital signs, forced vital capacity (FVC) and electrocardiogram (ECG) (if available in the participants' medical records within 12 months prior to the Screening Visit).

Data collected at study visits will be manually entered into the eCRF. The following data will be collected from participants at relevant time points for study assessments and procedures:

- Medical history (including biopsy details, if performed);
- Medication use (including all current and prior medications to treat symptoms of *RYR1*-RM);
- Full physical, neurological, and functional examination;
- Vital signs (heart rate, blood pressure, respiratory rate, temperature), FVC sitting and supine, and ECG;
- Strength measurements by QMA (shoulder abduction, elbow flexion and extension, knee flexion and extension), MMT (neck flexion), and HHD (neck flexion);
- 10 Meter Walk Test;
- 1-Minute Sit-to-Stand;
- Stair Climb Test; and
- PROMIS Fatigue, PROMIS-PF, IPAQ, and symptom diary.

Participants will be given periods of rest in between tests to reduce their burden. Ideally, all visits should be conducted in the morning to minimise the impact of the participant's daily activities on their energy levels, cognitive function, and mood. However, the actual timing of the visits may vary according to the capacity of the sites.

For details on data collected at each visit refer to the data collection schedule table in Section 4.5.

4.2 Study Population

The study will aim to enrol up to 20 participants from neurology clinics at study centres in Europe and the United Kingdom over a 2.5-month enrolment period.

4.2.1 Inclusion Criteria

The following criteria must be met to be enrolled in the study:

- Male and female patients (biological sex*) aged 18 years or older at Screening;
- Confirmed genetic diagnosis of *RYR1*-RM with autosomal dominant mutation and supporting clinical phenotype with demonstrable proximal weakness on at least one of the baseline study assessments;
- Evidence of at least one demonstratable muscle/motor function deficit assessed through Manual Muscle Testing (MMT) and scored using the Medical Research Council (MRC) Scale for muscle strength (17) on physical examination (see MRC scale described in Table 5);
- Able to walk 10 meters, with or without assistance - e.g., with a cane (assessed using the 10 Meter Walk Test);
- Willingness and ability to comply with scheduled visits and study procedures;
- Willingness to be fitted with the Syde® device at Screening Visit (for inclusion in the exploratory objective analysis only); and
- Able to provide written informed consent and understand the study procedures in the informed consent form (ICF).

*Biological sex of male or female is required for study inclusion based on the available normative values for the tests conducted, but all gender identities are eligible for inclusion in the study.

4.2.2 Exclusion Criteria

Patients meeting one or more of the following criteria will not be eligible for the study:

- Severe pulmonary dysfunction at Screening (FVC < 40% predicted) or evidence of pulmonary exacerbation (note that pulmonary exacerbations refer to acute worsening respiratory symptoms resulting from a decline in lung function);
- Significant cognitive impairment in the judgement of the investigator who will be unable to follow the protocol;
- Patients with progressive neurological conditions (e.g., Parkinson's disease);
- Non-ambulant patients; or
- Pregnant women.

4.2.3 Study Enrolment

The principal investigators (PIs) for the study will be neurologists who are routinely involved in the care and treatment of patients with *RYR1*-RM. Site selection criteria will include the projected availability of eligible patients in a 2.5-month enrolment period and the availability of the neurologist (and other site staff) to complete the assessments, case report forms (CRFs) and required study administration. Where possible, sites in each country specialised in treating RYR1-RM patients will be engaged in conducting the study. Selection criteria and basic site information (e.g., site size, investigator specialty, site type) will be collected via a site qualification survey.

Sites will assess initial eligibility for inclusion based on the patients' medical records (i.e., using information on their age, genetic diagnosis of *RYR1*-RM, and diagnosis of progressive neurological conditions). The eligibility criteria will be further assessed by a trained nurse/clinician during a telephone interview or as part of routine clinical care visits. For all eligible patients, the nurse/clinician will provide the Patient Information Sheet (PIS)/ICF to the patient for their review. If they agree to participate in the study, informed consent will be obtained from the patient (either independently or with the support of a caregiver, if required). Participants who consent to take part in the study will then be invited to attend the Screening Visit at the clinic (this may be conducted as part of their routine clinical care visits). At the Screening Visit, participants will be asked to confirm their consent to take part in the study (and sign the ICF if they have not done so already). Each site will maintain a Screening log to record the disposition of consecutive patients potentially eligible for study participation (e.g., demographic characteristics recorded in the patients' medical records), to better assess the representativeness of the sampled population.

4.2.4 Participant Withdrawal

Participants may withdraw consent and stop participating in the study at any time, with no effect on their medical care or access to treatment. If a participant withdraws prior to completing the study follow-up period, any known reason for withdrawal should be documented in the database. All information already collected as part of the study will be kept for analysis; however, no other data will be collected from the patient as a part of the study beyond the reason for discontinuation. A decision to replace withdrawn patients will be made on an ongoing basis based on the impact of the study's ability to deliver against its objective.

4.3 Exposure Definition and Measures

This protocol does not recommend the use of any specific treatments. No study medication is provided as part of participation. No restrictions on concomitant treatments are associated with

the study. All concomitant treatments will be carefully recorded to evaluate their potential influence on the outcomes of interest.

4.4 Outcome Definition and Measures

4.4.1 Endpoint Measures

4.4.1.1 Primary Endpoint Measures

Absolute measurements at each study visit and changes in muscle strength measured by QMA, HHD, and MMT will be assessed between Screening, each visit as well as between consecutive visits. QMA will be used to assess knee flexion and extension, elbow flexion and extension, and shoulder abduction. Neck flexion will be measured using HHD and MMT (**Error! Reference source not found.**). Additional tests that will be performed to measure muscle strength include the 10-MWT, 1-Minute-Sit-to-Stand Test, and Stair Climb Test. The Screening measurement will be the baseline for the study. All tests should be conducted at the same time of day and in a standardized order for all participants across the study sites as follows:

a) QMA;
b) HHD;
c) MMT;
d) 10-MWT;
e) 1-Minute-Sit-to-Stand Test; and lastly
f) Stair Climb Test.

Table 2. Method of assessment for muscle strength measurements

Method of assessment	Muscle movement
QMA[a]	Knee Extension
	Knee Flexion
	Elbow Extension
	Elbow Flexion
	Shoulder Abduction
HHD	Neck Flexion
MMT	Neck Flexion

HHD, Hand-Held Dynamometry; MMT, Manual Muscle Testing; QMA, Quantitative Muscle Assessment.

[a] For QMA, each muscle movement will be tested on both limbs (noting which is the dominant limb and starting with the dominant limb).

a) Quantitative Muscle Assessment (QMA)

QMA is a quantitative muscle testing (QMT) method that will be used to assess knee flexion and extension, elbow flexion and extension, and shoulder abduction. QMA is designed to measure Maximum Voluntary Isometric Contraction (MVIC), an objective and validated measure of muscle strength. MVIC has been adopted for the measurement of muscle strength in participants with neuromuscular disorders across several studies (18), and it has been shown to be a more reliable assessment tool than MMT methods. (19) MVIC can be measured using a strain gauge (which is attached to the participant on a standard plinth at one end and to orthopaedic bars at the other) or an HHD.

The QMA system consists of the following items (18, 20):

- QMA computer software package (Aeverl Medical, Gainesville, GA) (21), inclusive of a strain gauge, hand dynamometer, and data acquisition pad;
- QMT Frame;
- Standard double plinth; and
- Cuff and straps.

In this study, the QMA assessment of knee flexion and extension, elbow flexion and extension, and shoulder abduction will be conducted using a strain gauge. This method uses a fixed myometry assessment, namely a relative fixed point (i.e., frame) that the participant is instructed to exert maximum effort. (20) The testing procedure involves using an adjustable cuff to attach the participant's arm or leg to an inelastic strap that is connected to an adjustable frame with a load of 0.5 to 1,000 Newtons. The cuff is worn by the participant, and the participant is positioned on the plinth in such a way as to isolate the specific muscle movement under assessment. (22)

The QMA software package is a computer-based system that is designed to assist with the quantitative testing of muscle strength. This system converts the data generated through the test into a usable and comprehensible format and can generate reports about the results of the assessments. Detailed instructions for the use of the QMA software package can be found in the QMA user guide. (23, 24)

The QMA will be conducted at Screening, Visit 1, Visit 2, and End of Study Visit. Each muscle movement will be tested on both limbs (noting which is the dominant limb and starting with the dominant limb) and in a standardized order across sites. Three measurements per visit will be performed for each muscle movement, while the participant is verbally encouraged by the tester to exert maximal effort. For each trial, the maximum force that is generated by the

participant will be recorded. The highest score of the three measurements can be used to determine the final strength level of the muscle movement tested. (22)

The raw QMA scores are measured in Newtons and can be converted into Pounds or Kilograms. The raw scores can also be standardised and expressed as deviation from normative values derived from healthy individuals. (25) The individual and average values of the three measurements taken for each muscle movement will be presented as both raw scores and deviations from normative data in the statistical analysis (see Section 6.2).

Further information on the QMA procedure and muscle movements assessed is provided Table 3. (18)

Table 3. QMA procedure and muscle movements assessed

Muscle movement	Position of participant	Strap position	Stabilisation by tester	Instruction
Knee Extension	Sitting, hips and knees at 90˚	Proximal to ankle joint	Pressure through shoulder and pelvis	Straighten your knee. Do not hold the couch
Knee Flexion	Sitting, hips and knees at 90˚	Proximal to ankle joint	None	Bend your knee. Do not hold the couch
Elbow Extension	Supine, shoulder in neutral elbow in 90˚flexion	Proximal to wrist	Anterior surface of shoulder and lateral elbow	Straighten your elbow
Elbow Flexion	Supine, shoulder in neutral elbow in 90˚ flexion	Proximal to wrist	Anterior surface of shoulder and volar surface of elbow	Bend your elbow
Shoulder Abduction	Supine, shoulder in 90˚ abduction	Proximal to elbow	Axilla and lateral trunk	Bring your arm away from your body without lifting your arm off the bed

QMA, Quantitative Muscle Assessment.
Source: Meldrum et al. (18)

b) Hand-held Dynamometer (HHD)

Neck flexion will be assessed using a HHD, another valid and reliable QMT tool for objectively quantifying muscle strength. (26, 27) HHD is a portable device which measures the MVIC of specific muscle actions. HHD has been selected for measuring the MVIC of neck flexion because it is easier to assess this muscle movement through a hand-held device rather than straps as in QMA.

The procedure involves the participant pushing maximally against the device using a specific muscle movement for three to five seconds, while the tester applies a force to just overcome the strength of the participant, and the maximum force exerted by the muscle is recorded. (28) Each muscle action is assessed in a gravity-neutralized position. The specific testing position for testing neck flexion using HHD is described in Table 4.

Table 4. Position for the HHD measurement of neck flexion

Muscle movement	Position of participant	Dynamometer position
Neck flexion	Sitting upright; head up at 90˚ from horizontal	Centre of forehead, just above eyebrows

HHD, Hand-held dynamometry.
Source: van der Ploeg et al. (27)

HHD will be conducted at Screening, Visit 1, Visit 2, and End of Study Visit. Three measurements of neck flexion will be performed at each visit, and both the individual and average scores of the three measurements will be described. The raw scores are expressed in Newtons (and can be converted into Pounds or Kilograms) and will be compared against normative values derived from healthy individuals. (27)

c) Manual Muscle Testing (MMT)

Neck flexion will also be measured using MMT, another validated and reliable tool that is designed to manually assess muscle strength and function. (10, 29) This method involves testing the strength of specific muscle movements based on their performance in relation to the forces of gravity or manual resistance through the available range of motion. The strength applied by the participant is then graded using a validated MMT scale (e.g., 5-point MRC or 10-point Kendall scale). In this study, the MMT assessment of neck flexion will be scored using the 5-point MRC scale for muscle strength (Table 5). (30)

Table 5. MRC scale for the MMT assessment of neck flexion

Muscle function	Grade	Strength level
No muscle activation	0	Zero
Movement in horizontal plane		
Trace muscle activation, such as a twitch, without achieving full range of motion	1	Trace

Muscle activation with gravity eliminated, achieving full range of motion	2	Poor
Movement against gravity		
Muscle activation against gravity, full range of motion	3	Fair
Muscle activation against some resistance, full range of motion	4	Good
Muscle activation against examiner's full resistance, full range of motion	5	Normal

MMT, Manual Muscle Testing; MRC, Medical Research Council.

A description of the participant's and tester's position and procedure for assessing neck flexion using MMT is provided in Table 6. Of note, the test involves both an anti-gravity position and a gravity-eliminated position. The anti-gravity position should be tested first for all participants, while the gravity-eliminated position should only be tested in weaker participants who cannot move against gravity. (31) Further information about the test procedure can be found in the MMT manual. (32)

Table 6. Position and procedure of the MMT assessment of neck flexion

Position in relation to gravity	Position of participant	Position of tester	Procedure
Anti-gravity	Supine, with arms by the side and head supported on a table	Standing next to the participant's head, with the testing hand placed on the participant's forehead	The participant lifts their head off the table by flexing the neck and tucking the chin. The tester applies resistance at the forehead in the direction of capital and cervical extension and may position a hand underneath the subject's head for protection or offer additional stabilization across the abdomen (if needed)
Gravity-eliminated (for weaker participants only)	Side lying, with head supported on the table and arms by the side	Supporting the participant's head to prevent cervical side bending, and providing stabilization at the anterior shoulder as needed	Participant is instructed to flex their head and neck towards their chest

MMT, Manual Muscle Testing.
Source: MMT manual (32).

The MMT assessment of neck flexion will be conducted at Screening, Visit 1, Visit 2, and End of Study Visit. Three measurements of neck flexion will be performed at each visit, and both the individual and average scores of the three measurements will be described.

Although both MMT and HHD are designed to measure the same underlying concept, the literature only suggests moderate correlations between their measurements of neck flexion. (10) Such discrepancy may be explained by the fact that MMT has decreased sensitivity and specificity in detecting mild weakness compared to HHD. Furthermore, the grading system of MMT is subjective and may vary according to the strength of the examiner. (33, 34)

d) 10 Meter Walk Test (10-MWT)

This test will be used to assess walking speed in meters/second (m/s). A clear pathway of at least 10 meters (32.8 ft) in length in a designated area over solid flooring and a stopwatch will be required. A mark at 2m and 8m (identifying the central 6m which will be timed) will be

marked. The total time taken to ambulate 6m will be recorded to the nearest hundredth of a second. 6m is then divided by the total time (in seconds) taken to ambulate and will be recorded in m/s. Two trials of the test should be conducted at the participant's comfortable walking speed, followed by two additional trials at their fast walking speed. (35)

In order to score the test, the time is measured for the middle 6m to allow for participant acceleration and deceleration. The tester should start recording the time when any part of the leading foot crosses the plane of the 2m mark, and the time is then stopped when any part of the leading foot crosses the plane of the 8m mark. (35)

The time to walk the middle 6m, the level of assistance, and type of assistive device and/or bracing used will be documented. If a participant requires total assistance or is unable to ambulate at all, a score of 0 m/s should be documented. The level of physical assistance is scored using an ordinal 7-point scale, as follows:

- 1 = total assistance (participant performs 0-24% of test);
- 2 = maximum assistance (participant performs 25-49% of test);
- 3 = moderate assistance (participant performs 50-74% of test);
- 4 = minimum assistance (participant performs 75-99% of test);
- 5 = supervision (participant requires stand-by or set-up assistance; no physical contact is provided);
- 6 = modified independent (participant requires use of assistive devices or bracing, needs extra time, mild safety issues); or
- 7 = independent.

The 10-MWT will be conducted at Screening, Visit 1, Visit 2, and End of Study Visit. The individual and average raw scores of each trial (expressed as m/s), as well as their deviation from normative values in healthy individuals (36), will be described.

e) 1-Minute Sit-to-Stand Test

The 1-Minute Sit-to-Stand Test will be used as an additional validated measure of the participants' muscular weakness. (12, 13) This test requires an armless chair, and the participant is instructed to perform as many sit-to-stand actions as possible in 1 minute without using the upper limbs.

The 1-Minute Sit-to-Stand Test will be administered at Screening, Visit 1, Visit 2, and End of Study Visit. Participants will undertake three trials at each visit. The number of sit to stands completed in one minute will be described for each trial (both individual and average scores per visit), along with their deviation from normative values. (12)

f) 4 Stair Climb Test (4SCT)

Lastly, the 4SCT will be used to assess leg muscle power. The test equipment consists of a stopwatch and a flight of 4 stairs with rail. The participant will stand at the base of the stairs and will be required to ascend the stairs as fast as possible. Timing will start after the tester instructs the participant to start climbing and will stop when both participant's feet reach the top step. (15, 37)

This test will be administrated at Screening, Visit 1, Visit 2, and End of Study Visit, including three trails per visit. Stair climb power will be calculated using the following formula: power = [(body weight in kg) x (9.8 m/s^2) x (stair height in meter)]/(time in seconds). (15, 37) The raw scores of the 4SCT will be expressed as Watts and will also be described as deviations from normative values. (38)

4.4.1.2 Secondary Endpoint Measures

The secondary endpoint measures evaluated in this study include a) the PROMIS Fatigue, PROMIS-PF, and IPAQ questionnaires, b) demographics and clinical characteristics of participants, and c) symptom diary.

a) PROMIS Fatigue, PROMIS-PF, and IPAQ questionnaires

PROMIS Fatigue (PROMIS-F): The PROMIS-F tool is a National Institutes of Health (NIH) developed Patient Reported Outcomes Measurement Information System (PROMIS) used to measure fatigue across a wide range of chronic health conditions. It has been used extensively and clinically validated across diverse chronic disorders. (39, 40). It can be administered as a Computerised Adaptive Test (CAT) or in various fixed length short forms. The PROMIS Short Form v1.0 Fatigue 7a (PROMIS F-SF 7a) is a reliable and valid 7-item questionnaire which has been evaluated across various populations, and it will be used to assess fatigue in this study (see Appendix 11.2). (41, 42) Using this measure, the participant is expected to respond to questions on symptoms over a period of 7 days prior to administration of the form. Each question has five response options ranging in value from one to five. Scores are represented as 'Never'-1, 'Rarely'-2, 'Sometimes'-3, 'Often'-4, and 'Always'-5. A total raw score for the short form with all questions answered is a sum of the values of the response to each question. The lowest possible raw score is 7; the highest possible raw score is 35. Using a score conversion table provided in the user manual, raw scores are translated into a T-score for each participant. The T-score rescales the raw score into a standardized score with a mean of 50 and a standard deviation (SD) of 10. (42) Higher PROMIS T-scores indicate higher fatigue. A PROMIS T-

score higher than 50 represents more fatigue than the general population. Conversely, a T-score lower than 50 indicates less fatigue than the average. All questions must be answered in order to produce a valid score using the scoring tables provided in the measure specific scoring guide. For any unanswered questions, the Health Measures Scoring Service can be used to generate a final total score for participants with missing responses. The Health Measures Scoring Service is a web-based application that scores an Excel file of raw participant responses and returns by email a file with calculated T-scores for all measures including fields with missing responses. It is free to use but requires registration on the site in order to download an Excel file to input responses from the specific PROMIS measure and upload onto the service for the T-scores to be calculated. (42) The approach for dealing with the possible presence of item-level missing values in the data collected through the questionnaires will be further outlined in the SAP.

PROMIS Physical Function (PROMIS-PF): The PROMIS-PF is used to measure physical functioning and is efficient and sensitive to changes across a broad range of functioning in chronic disease conditions. The PROMIS-PF can be administered as a CAT, or as a stand-alone Short Form. In this study, the 10-item PROMIS Short Form v2.0 Physical function 10a (PROMIS PF-SF 10a) will be used (Appendix 11.3). (43) This short form has been validated in similar populations in previous studies. (40) It includes 10 questions, with five possible responses for each question. For the first set of five questions, response options are represented as 'Not at all' -5, 'Very little'- 4, 'Somewhat' -3, 'Quite a lot' 2, and 'Cannot do' -1. For the second set of five questions, the response options are, 'Without any difficulty' -5, 'With a little difficulty' - 4, 'With some difficulty' -3, 'With much difficulty' -2, and 'Unable to do' -1. The lowest possible score is 10 and the highest possible score is 50. (44) This scale can be scored either using the Health Measures Scoring Service, a data collection tool to automatically calculate participant scores, or using raw summed scores as detailed in the measure specific scoring guide. (44) The raw summed scores method will be used in this study. For this measure, as with the PROMIS F-SF, a total raw score for the short form with all the questions answered will be calculated for each participant and converted to a T-score using a score conversion table provided in the user manual. (44)

International Physical Activity Questionnaire (IPAQ): This questionnaire is a self-reported measure of physical activity and has been validated in a previous multi-country study. (45) The questionnaire is divided into domains with questions on vigorous physical activity, moderate physical activity, time spent walking, and time spent sitting over the previous 7-day time period. The IPAQ Short Form will be used in this study (IPAQ-SF; see Appendix 11.4). Two forms of output can be generated from scoring the IPAQ. Results can either be reported in categories (low , moderate or high activity levels) or as a continuous variable indicating Metabolic Equivalent (MET) minutes a week. Physical activity levels will be described as both continuous (in MET minutes per week) and as categories (low, moderate and high) in this study. MET

minutes represent the amount of energy expended carrying out physical activity. One MET is what is expended when an individual is at rest, and two METS is twice what is expended at rest. To get a continuous variable score from the IPAQ (MET minutes a week), walking is considered to be 3.3 METS, moderate physical activity 4 METS, and vigorous physical activity 8 METS. (46) Scores are categorised into three categories (high, moderate and low). The specific criteria used for scoring the IPAQ are showed in Table 7. (46)

Table 7 Scoring for International Physical Activity Questionnaire (46)

Scoring Categories	Criteria
High	Vigorous intensity activity on at least 3 days achieving a minimum total physical activity of 1500 MET minutes a week. OR 7 or more days of any combination of walking, moderate intensity or vigorous intensity activities achieving a minimum of at least 3000 METS minutes a week.
Moderate	3 or more days of vigorous intensity activity and/or walking of at least 20 minutes per day. OR 5 or more days of moderate intensity activity and/or walking for a minimum duration of 30 minutes per day. OR 5 or more days of any combination of walking, moderate intensity or vigorous intensity activities achieving a minimum total physical activity of at least 600 MET minutes a week.
Low	If criteria for *high* or *moderate* categories are not met.

To calculate MET minutes in a week, the MET value given number (walking = 3.3, moderate activity = 4, vigorous activity = 8) is multiplied by the minutes the activity was carried out for and by the number of days that that activity was undertaken. For example, if someone reports walking for 30 minutes 5 days a week, then the total MET minutes for that activity are 3.3 x 30 x 5 = 495 MET minutes a week). (46) MET minutes achieved in each category (low, moderate, and high activity) can be summed to get total MET minutes of physical activity per week.

The PROMIS-F, PROMIS-PF, and IPAQ questionnaires will be completed by the participants at Screening and End of Study visits. Details on domains measured using the above mentioned questionnaires are given in

Table 8.

b) Demographics and clinical characteristics

Demographics and clinical characteristics that will be assessed in the study include sex, year of birth, medication use (current and prior medications), medical history, height, weight, BMI, vital signs, FVC, ECG, and full physical, neurological, and functional examination. The tests that will be conducted as part of the physical, neurological, and functional examination are part of routine care for participants with *RYR1*-RM and are outlined in Appendix 11.111.1.

All the above demographics and clinical characteristics will be assessed at the Screening Visit. Medication use will also be assessed at Visit 1, Visit 2, and End of Study Visit, and the physical, neurological, and functional examination will be repeated at the End of Study Visit.

c) Review of symptom diary

A symptom diary will be completed by participants to collect data on their subjective experience of permanent and intermittent symptoms of the disease, as well as medications and other strategies used to manage their symptoms (including healthcare resource utilisation). Participants will record symptoms in the diary on a continuous basis, starting from the Screening Visit until the End of Study Visit.

Table 8 Questionnaires

Measures	Dimensions
PROMIS-Fatigue SF v1.0 7a	• Frequency of feeling tired • Frequency of experiencing extreme exhaustion • Frequency of running out of energy • Frequency of fatigue limiting to work (include work at home) • Frequency of feeling too tired to think clearly • Frequency of feeling too tired to take a bath or shower • Frequency of having enough energy to exercise strenuously
PROMIS-PF-SF 10a Item Short Form (Covers Self-care and Lower Extremity)	• Being able to dress oneself, including tying shoelaces and doing buttons • Being able to shampoo hair • Being able to wash and dry body • Being able to get on and off the toilet • Being able to do chores such as vacuuming or yard work • Health limiting in bending, kneeling, or stooping • Health limiting in lifting or carrying groceries • Health limiting in climbing one flight of stairs • Health limiting in walking more than a mile • Health limiting in doing vigorous activities
Additional Item on Physical Function	• Do you have difficulties turning in bed or moving in bed?

Measures	Dimensions
IPAQ 7 Item Short Form (covers past 7 days)	• Number of days doing vigorous physical activities like heavy lifting, digging, aerobics, or fast bicycling • Amount of time per day doing those vigorous physical activities • Number of days doing moderate physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis • Amount of time per day doing those moderate physical activities • Number of days walking for at least 10 minutes at a time • Amount of time per day spending walking • Amount of time per day spending sitting

IPAQ, International Physical Activity Questionnaire, PROMIS, Patient-Reported Outcomes Measurement Information System; PROMIS, Patient-Reported Outcomes Measurement Information System; PROMIS-PF, Patient-Reported Outcomes Measurement Information System-Physical Function.

4.4.2 Safety Reporting

Any adverse event/serious adverse event (AE/SAE) will be recorded throughout the study. Details of AE/SAE that will be collected are specified in Section 8.

4.4.3 Other Measures

Explorative endpoints include measurements obtained using the Syde® device to objectively assess activity (e.g., stride velocity when walking and climbing stairs) from Screening Visit (Syde® device fitting) to Visit 1 (Syde® device removal).

4.5 Data Sources and Collection

Data will be collected during visits to the site and extracted from the participants' medical records (paper and/or electronic) at the Screening Visit. The data collected at study visits will be manually entered in an eCRF. The relevant data time points for the study are presented in the data collection schedule in

Table 9. The Screening Visit is anticipated to take approximately 1 hour and 30 minutes, while subsequent visits should take approximately 1 hour.

Table 9. Data Collection Schedule

Study Procedure	Screening Visit (0 day)	Visit 1 (+30 days [±3 days])	Visit 2 (+30 days [-14/+3 days])	End of Study Visit (+30 days [-14/+3 days])
Informed consent	X	-	-	-
Inclusion/Exclusion	X	-	-	-
Demographics	X	-	-	-
Medical history [a]	X	-	-	-
Medication use [b]	X	X	X	X
Strength measurements using QMA, HHD and MMT [c]	X	X	X	X
10-MWT	X	X	X	X
1-Minute Sit-to-Stand Test	X	X	X	X
4SCT	X	X	X	X
PROMIS Fatigue	X	-	-	X
PROMIS-PF + additional single item	X	-	-	X
IPAQ	X	-	-	X
Vital signs (HR, BP, RR, temperature), FVC, and ECG [d]	X	-	-	-
Full physical, neurological, and functional examination [e]	X	-	-	X
Syde® device fitting and removal	X	X	-	
Symptom diary [f]	X	-	-	X
AE/SAE	X	X	X	X

[a] Medical history: Include if a biopsy has been performed and capture related data.
[b] Medication use: To include all current medication and prior medication to treat symptoms of *RYR1*-RM.
[c] Strength measurements: Shoulder abduction, neck flexion, elbow flexion and extension, knee extension and flexion.
[d] Vital signs, FVC and ECG will be assessed at the time of the Screening Visit or based on the most recent assessment captured in the participants' medical records within 12 months prior to the Screening Visit.
[e] See list of tests in Appendix 11.1.
[f] Participants will keep a diary of their symptoms on a continuous basis throughout the study, starting from the Screening Visit up to End of Study Visit.
10-MWT, 10-Minute Walk Test; 4SCT, 4 Stair Climb Test; AE, adverse event; BMI, body mass index; BP, blood pressure; ECG, electrocardiogram; FVC, forced vital capacity; HR, heart rate; IPAQ, International Physical Activity questionnaire; PROMIS, Patient-Reported Outcomes Measurement Information System; PROMIS-PF, Patient-Reported Outcomes Measurement Information System-Physical Function; RR, respiratory rate; *RYR1*-RM, Ryanodine receptor isoform 1-related congenital myopathies; SAE, serious adverse event.

4.5.1 Screening Visit

The following data will be collected at the Screening Visit for all enrolled participants (listed in the order in which they will be collected during the visit):

- Informed consent;
- Inclusion/Exclusion (including MMT to assess muscle/motor function deficit, 10-MWT to assess ability to walk, and FVC to assess pulmonary dysfunction);
- Demographics (medical records);
- Medical history (medical records) ;
- Medication use (medical records);
- Vital signs and ECG (medical records or primary data collection; note that FVC will be evaluated as part of the assessment of the exclusion criteria);
- Strength measurements using QMA and HHD (note that MMT will be conducted at the start of the Screening Visit as part of the assessment of the inclusion criteria);
- 1-Minute Sit-to-Stand Test;
- 4SCT;
- Full physical, neurological, and functional examination;
- PROMIS Questionnaires;
- IPAQ;
- Syde® device fitting;
- Symptom diary (initiation); and
- AE/SAE.

4.5.2 Visit 1, Visit 2, and End of Study Visit

The following data will be collected for all enrolled participants at each follow-up time point:

- Medication use;
- Strength measurements using QMA, HHD, and MMT;
- 10-MWT;
- 1-Minute Sit-to-Stand Test;

- 4SCT;
- PROMIS Questionnaires (at End of Study Visit only);
- IPAQ (at End of Study Visit only);
- Full physical, neurological, and functional examination (at End of Study Visit only);
- Syde® device removal (at Visit 1 only);
- Symptom Diary (to be completed daily between Screening Visit and End of Study Visit); and
- AE/SAE.

4.5.3 4.5.3 Study Withdrawal/Discontinuation

The following data will be collected for all enrolled participants at the time of discontinuation:

- Date of discontinuation; and
- Reason for discontinuation.

Once the participant informs the PI that they wish to withdraw or discontinue from the study, then no additional information or data will be captured for that participant.

4.5.4 End of Study

The End of Study is defined as the date of the last query resolution i.e all data has been recorded in the electronic case report form and all data queries resolved to allow database lock to occur. This will ensure that all data is available to answer the research questions in the study protocol. This definition will be used across all participating sites.

5. DEVICES

The use of digital tools, such as wearable devices and other remote sensors, allows for the continuous, objective, and sensitive measurements of a participant's functional ability during daily life. Validated class I medical wearable devices have been used in clinical trials for assessing functional outcomes in daily life for participants with neuromuscular disorders (48) including clinical trials of participants with Duchenne Muscular Dystrophy and can be used in both ambulant and non-ambulant participants with movement disorders. (49)

Participants will be provided with a wearable device to collect daily physical activities. The purpose of the wearable device is to accurately measure the movement and activity levels of

the participant during normal daily living, outside of investigational site visits. The Syde® device consists of 2 sensors (and a docking station). It is a Class I medical device manufactured by Sysnav. There are no foreseeable risks and minimal inconveniences to the participants who will wear it. Participants will wear one sensor on each ankle.

As the wearable device battery requires recharging daily after use, the sensors will not be worn at night. Site personnel, the participant, and parents/caregivers will be trained on the correct use of the device. The device will be fitted on the participant in the clinic prior to the Stair Climb Test, in order to ensure that it is working well, which can be assessed as participant climbs the stairs. The device would be removed when participants take part in activities which could damage the sensors or where the device could be in contact with water. Detailed user instructions are provided in the wearable device participant manual and quick start guide.

The wearable device will be worn on both ankles daily for 30 (±3) days (to be taken off only at night before sleeping) from Screening Visit [Syde® device fitting] to Visit 1 [Syde® device removal]).

The quantity of data recording will be closely monitored by Sysnav and communicated to the sites to maintain an adequate participant compliance throughout the recording period, until the end of the study. The recorded data will then be checked for accuracy, completeness, relevance, and consistency, before the last step of data analysis which includes the process of using algorithms to generate the Syde® clinical variables.

The wearable device will not be worn during clinic visits, but participants will resume use immediately following completion of in-clinic tests. Subjects must return the wearable device in the event of screen failure, early termination, or after study completion.

6. STATISTICAL METHODS

6.1 Sample Size

The study plans to enrol up to 20 participants. This sample size is based on the number of participants that will be feasible to recruit at the participating sites. Given the explorative and descriptive nature of this study and the rarity of the target population, formal sample size calculations were not performed. Accordingly, the primary focus of the study is on measuring and describing the study endpoints in the target population rather than on statistical hypothesis testing.

6.2 Data Analyses

6.2.1 General Considerations

All computations and generation of tables, listings, and data for figures will be performed using SAS® version 9.2 or higher (SAS Institute, Cary, NC, USA).

Prior to database lock, a detailed, finalized SAP will be completed and approved. The SAP will contain a more comprehensive description of the methodology for the statistical analyses than outlined in this protocol. The SAP will provide full details of the analyses, the data displays, and the algorithms to be used for data derivations.

Continuous variables will be described using descriptive statistics of central tendency (median and mean) and dispersion (standard deviation, interquartile range, maximum and minimum values). Categorical variables will be summarized as counts and proportions of the total study population. The 95% confidence interval will be presented for estimates of means and proportions. Descriptive statistics for both continuous and categorical variables will be based on participants with non-missing responses to the variable. Frequencies and percentages of missing values in each variable will be reported.

Whenever applicable, the endpoints will be summarized by each visit and across the overall study period (e.g., average endpoint across study visits). Furthermore, the absolute change in the endpoints between (i) Screening and each follow-up visit, and (ii) between each consecutive follow-up visit will be calculated and summarized with descriptive statistics.

6.2.2 Planned Analyses

6.2.2.1 Primary Objective Analysis

Muscle strength endpoints measured through the QMA, HHD, and MMT and functional test scores (including 10-MWT, 1-Minute-Sit-to-Stand Test, and 4SCT) will be summarized using descriptive statistics, as detailed in Section 6.2.1 (including absolute values and changes). These endpoints will be described as both raw scores and deviations from normative values in healthy individuals (if available).

The variability of the primary study endpoints between repeated measurements and/or visits will be assessed using graphical methods and correlation coefficients.

Further details about the statistical methodology, including any comparison to available normative data will be included in the SAP.

6.2.2.2 Secondary Objective Analysis

The PROMIS Fatigue, PROMIS-PF, and IPAQ questionnaires will be scored according to their manual instructions (see Section 4.4.1.2). PRO endpoints assessed through the PROMIS Fatigue, PROMIS-PF, and IPAQ questionnaires and the symptom diary will be summarized using appropriate descriptive statistics, as outlined in Section 6.2.1.

Demographic and clinical characteristics of participants and data collected through the diary will be summarized with descriptive statistics, as outlined in Section 6.2.1. AE endpoints recorded throughout the study will also be summarized through descriptive analysis.

6.2.2.3 Explorative Objective Analysis

Explorative endpoints will be summarized using descriptive statistics, as appropriate.

Additional exploratory analyses may be performed and outlined in the SAP prior to the data analysis.

6.2.3 Handling of Missing Data

It is optimal to prevent missing data, to the extent possible, through strategies set forth in the design and conduct of a study. For the current study, we will aim to minimize missing information by:

- Ensuring that primary variables of interest are those that are routinely collected as part of real-world clinical care and are available via medical charts, physician and/or participant/caregiver reporting, as appropriate;
- Collecting only critical data elements (i.e., variables aligned with the study objectives) to minimize site/participant burden;
- Including “not applicable” or “not done” on CRFs to differentiate missing values; and
- Training of sites and data abstractors regarding data collection and setting reporting windows around a target time point.

Should missing data occur, the data will be analysed as they are recorded in the study database. The number of missing values for data elements will be reported, and the impact of missing data on the analysis and the pattern of the missing information will be assessed. Full details on the handling of missing data will be described in detail in the SAP (e.g., approach to dealing with missing data on the individual questionnaire items in the scoring of the questionnaires).

6.2.4 Limitations of Research Methods

6.2.4.1 Selection Bias

Given the small size of the study and the use of a non-random sampling approach, the recruited sample of participants may not be representative of the target population; hence, the results may not be generalizable to the wider population.

6.2.4.2 Confounding Bias

Due to the observational nature of the study, any observed changes in the endpoints or relationships between different study endpoints could be explained by unmeasured confounding factors not accounted for in the analysis.

6.2.4.3 Information Bias

The presence of missing data may introduce bias to the study results. To mitigate this bias, the patterns of missing data will be analysed, and potential imputation approaches will be discussed with the study team, depending on the nature and quantity of the missing information.

Self-report bias (e.g., social desirability, recall bias) could affect the PRO measures. This risk will be mitigated by using validated questionnaires, whenever possible, and by providing detailed instructions and guidance to participants on the completion of the PRO questionnaires.

Measurement bias, which could result from variations in the weight of the participants' extremities, the force applied by the examiner, and the strength of the examiner, could affect the measurement of the endpoints. This will be mitigated by ensuring adequate training for examiners taking the measurements.

Systematic errors in reporting data into the eCRFs could be another source of measurement bias. This will be mitigated through implementing rigorous data quality and monitoring procedures for the data collected by the eCRF and by providing rigorous training to the study team.

6.2.4.4 Statistical Power

The study has limited power to detect statistical differences due to the small size of the sample. Any confidence intervals and p-values from statistical hypothesis tests will be considered descriptive and should be interpreted with caution.

6.3 Data Reporting

6.3.1 Progress Reports

Not applicable.

6.3.2 Annual/Interim Analyses and Reporting

As this is a non-interventional observational study the data will be assessed on an ongoing basis for accuracy and completeness, however full reporting will only be done when all participants have completed, and the study is considered closed.

6.3.3 Final Analyses and Reporting

A final study report will be generated after all data collection is complete. The final report will encompass all planned analyses, including a description of the complete study population, as described above and in the SAP.

7. STUDY MANAGEMENT

This study will be performed by IQVIA with guidance, input, review, and approval of the Sponsor., including development of materials, recruitment, training and management of sites, electronic data capture (EDC) and data management and analysis.

7.1 Data Entry/Electronic Data Capture

All data will be collected and entered directly into the EDC system. Data for the study may be entered directly into the EDC system by the study staff or pre-populated using electronic health record data and later verified by the study staff.

All sites will be fully trained in using the online data capture system, including eCRF completion guidelines and help files. Sites will manage entering extracted participant data into a secure internet-based EDC registry database via the eCRF. Investigators and site personnel will be able to access their account with a username and password. All eCRFs should be completed by designated, trained personnel or the study coordinator, as appropriate. In most cases, the eCRF should be reviewed, electronically signed, and dated by the principal investigator. All changes or corrections to eCRFs are documented in an audit trail and an adequate explanation is required.

Syde® will have 2 watch-like devices. The watch-like devices contain a three-axis accelerometer, a three-axis gyroscope, and a three-axis magnetometer that record, respectively, the linear acceleration, the rotation rate (angular velocity), and the magnetic field in the 3 dimensions of space. These sensors are just plugged on the docking station every night by

participants. If a participant authorizes automatic data upload, then data gets uploaded automatically to Sysnav cloud storage. If a participant does not authorize automatic data upload, or if automatic data upload is not possible then data gets stored on an encrypted USB drive within the docking station, retrieved by the clinical site at the end of the recording period and shipped back to Sysnav. It is then uploaded by Sysnav authorized personnel to the cloud.

7.2 Source Documents

In most cases, the source documents are contained in the participant's medical record and data collected on the CRFs must match the data in the medical records. In some cases, the CRF, or part of the CRF, may also serve as source documents. In these cases, a document should be available at the investigator's site and clearly identify those data that will be recorded in the CRF, and for which the CRF will stand as the source document. All original source documentation is expected to be stored at the site for the longest possible time required by applicable local regulations.

7.3 File Retention and Archiving

To enable evaluations and/or audits from regulatory authorities or the Sponsor, the investigator agrees to keep records, including the identity of all participating participants, all original signed ICFs, copies of all CRFs, SAE forms, source documents and adequate documentation of relevant correspondence (e.g., letters, meeting minutes, telephone calls reports).

Each site will receive a study site file at study initiation which contains all documents necessary for the study and is updated throughout the study. This file must be available for review in the event the site is selected for monitoring, audits, or inspections and must be safely archived for at least 15 years after the completing participation in the study unless local regulations require a longer archival period. No records can be destroyed during this time without agreement from the Sponsor and no records may be transferred without agreement from the Sponsor. Documents to be archived include the participant enrolment log and the signed ICF. If archiving of the file is no longer possible at the site, the site will be instructed to notify the Sponsor.

7.4 Quality Assurance and Monitoring

A study quality assurance and monitoring plan, which is proper for the study design, will be developed and implemented.

During the remote site initiation visit, the monitor will provide training on the conduct of the study to the investigator, co-investigator(s), and all site staff involved in the study. To ensure the integrity of the data, sites will be monitored. Remote site monitoring will be performed by IQVIA CRAs or Sponsor representatives to examine compliance with the protocol and

adherence to the data collection procedures, to assess the accuracy and completeness of submitted clinical data and to verify that records and documents are being properly maintained for the duration of the study. The monitor will perform source data verification by reviewing the original participant records.

The monitor will close out each site remotely after the last participant's final follow-up assessment is completed, all data have been entered and all outstanding monitoring issues have been resolved or addressed. All monitoring procedures and the frequency of monitoring visits will be described in a monitoring plan. Monitor contact details for each participating site will be maintained in the Investigator Site File.

Representatives of the Sponsor's quality assurance unit/monitoring team and competent regulatory authorities must be permitted to inspect all study-related documents and other materials at the site, including the Investigator Site File, the completed eCRFs and the participants' original medical records. Audits may be conducted at any time during or after the study to ensure the validity and integrity of the study data.

7.5 Data Management

A data management plan will be created before data collection begins and will describe all functions, processes, and specifications for data collection, cleaning, and validation. The eCRFs will include programmable edits to obtain immediate feedback if data are missing, out of range, illogical or potentially erroneous. A concurrent manual data review will be performed based on parameters dictated by the plan. Ad hoc queries will be generated within the EDC system and followed up for resolution.

High data quality standards will be kept, and processes and procedures will be used to repeatedly ensure that the data are as clean and correct as possible when presented for analysis. Data quality will be enhanced through a series of programmed data quality checks that automatically detect out of range or anomalous data.

7.6 Changes to the Protocol

Changes to the protocol will be documented in written protocol amendments. Major (i.e., substantial, significant) amendments will usually require submission to the relevant institutional review board (IRB)/independent ethics committee (IEC) for approval or favourable opinion. In such cases, the amendment will be implemented only after approval or favourable opinion has been obtained.

Minor (non-substantial) protocol amendments, including administrative changes, will be filed at each participating site, and will be submitted to the relevant IRB/IEC or regulatory authorities where required by pertinent regulations. Any amendment that could impact the participant's

agreement to participate in the study requires their informed consent prior to continued participation.

7.7 Study Governance

Not applicable.

7.8 Publication Policy

Any publication of the results from this study must be consistent with the Sponsor's publication policy and guided by the Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication of the International Committee of Medical Journal Editors, updated April 2010. The rights of the investigator and of the Sponsor about publication of the results of this study are described in the applicable investigator contract with Sponsor.

All reporting will be consistent with the STROBE (Strengthening the Reporting of Observational studies in Epidemiology) Initiative checklist for cohort studies STROBE 2008.

8. SAFETY REPORTING

All AEs, regardless of their relationship to use of the Syde® medical device or study procedures will be monitored and reported throughout the study. The AE reporting period begins when the participant is included into the study (date of first signature of informed consent) and continues till the end of study as defined by the Sponsor. AEs will be recorded on the appropriate forms (e.g., CRFs, eCRFs) as designated by the Sponsor.

8.1 Definitions

Adverse events

An AE is any untoward medical occurrence in a participant or clinical study subject that is administered a medical device or undergoing a study procedure and does not necessarily need to have a causal relationship. An AE can therefore be any unfavourable and unintended sign symptom, or disease temporally associated with the use of a device or study procedure, whether considered related to the medical device or study procedure or not.

If, according to the investigator, there is a worsening of a medical condition that was present prior to the exposure to a medical device or study procedure, this should also be considered a new AE and reported. Any medical condition present prior to the administration of the Syde® medical device or study procedure that remains unchanged or improved should not be recorded as an AE at subsequent visits.

Documentation regarding the AE should be made as to the nature, date of onset, end date, severity, relationship to Syde® medical device or study procedure, action(s) taken, and outcome of any sign or symptom observed by the physician or reported by the participant upon indirect questioning.

Serious adverse events

An SAE is any experience that suggests a significant hazard, contraindication, side effect or precaution. An SAE must fulfil at least one of the following criteria:

- Results in death;
- Is life-threatening as it occurred (participant was at risk of death at the time of the event; this does not refer to an event which might have caused death if it were more severe);
- Requires inpatient hospitalization or prolongation of existing hospitalisation;
- Results in persistent or significant disability/incapacity (defined as a substantial disruption of a participant's ability to conduct normal life functions);
- Results in a congenital anomaly or birth defect; or
- Constitutes an important medical event (based upon appropriate medical judgment, event may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above).

Event severity

Event severity is defined as a qualitative assessment of the degree of intensity as determined by the investigator or reported to them by the participant. The assessment of severity is made irrespective relationship or seriousness of the event and should be evaluated according of the following scale:

- Mild: The event is noticeable to the participant, but is easily tolerated, and does not interfere with the participant's daily activities;
- Moderate: The event is bothersome, requiring additional therapy, and may interfere with the participant's daily activities;
- Severe: The event is intolerable, necessitates additional therapy or alteration of therapy, and interferes with the participant's daily activities.

Note: The terms "severe" and "serious" are not synonymous. Severity refers to the intensity of an AE (as in mild, moderate, or severe pain); the event itself may be of minor medical

significance (such as severe headache). "Serious" is a regulatory definition and is based on participant or event outcome or action criteria usually associated with events that pose a threat to a participant's life or vital functions.

Relationship to study procedure

For all events reported in participants exposed to the Syde® medical device or study procedure, the treating physician or other reporting health care provider will be asked to assess the relationship of the AE/SAEs to the Syde® medical device or study procedure using the following definitions:

- Probable: A causal relationship is clinically/biologically highly plausible, and there is a correlation between the onset of the AE/SAE and administration of the Syde® medical device or study procedure and resolution of the AE/SAE;
- Possible: A causal relationship is clinically/biologically plausible and there is a correlation between the onset of the AE/SAE and administration of the Syde® medical device or study procedure;
- Unlikely: A causal relationship is improbable, and another documented cause of the AE/SAE is most plausible;
- Unrelated: A causal relationship can be definitively excluded, and another documented cause of the AE/SAE is most plausible.

8.2 Procedures for Reporting AEs in the Case Report Form

All AEs (serious and non-serious) reported during the study visits will be captured on the appropriate study CRF including the description, seriousness criteria, severity, duration (onset and resolution date), causal relationship with the Syde® medical device or study procedure, actions taken, and outcome.

The outcome of each AE (serious or non-serious) should be entered with a term such as those described below:

- recovered without sequelae
- recovered with sequelae
- ongoing
- change in severity grade (worsening, improving)
- died

If any of the same AEs occur on several occasions in the same participant, then the AE in question must be documented and assessed each time.

8.3 Procedures for Reporting Adverse Events (AEs) to Local and Regional Health Authorities

In addition to recording the event on the CRF, all SAEs and non-serious AEs considered related to the Syde® medical device or study procedure, reported during study visits may also need to be reported to local health authorities and regional bodies as per local guidelines. The participating physician is responsible for maintaining compliance with local guidelines as well as with any applicable site-specific requirements related to the reporting of SAEs or other safety information to the local IRB/IEC that approved the study.

9. ETHICAL AND REGULATORY CONSIDERATIONS

9.1 Guiding Principles

To ensure the quality and integrity of research, this study will be conducted under the guidelines for good pharmacoepidemiology practices issued by the International Society for Pharmacoepidemiology, the Declaration of Helsinki and its amendments, and any applicable national guidelines.

9.2 Required Documents

Prior to the enrolment of any participants in the study, the following documents must be provided by the site to the Sponsor (or their designee):

- Copy of the IRB/IEC approval letter for the protocol and informed consent (all written information provided to the participant must be approved by the IRB/IEC);
- Copy of the IRB/IEC approved informed consent document to be used;
- Copy of the protocol signature page signed by the investigator; and
- Fully executed site agreement with Sponsor.

9.3 Patient Information and Informed Consent

An ICF must be signed by the participant before his or her participation in the study. The medical file for each participant should document the informed consent process and that written

informed consent was obtained prior to participation in the study. A copy of each signed ICF must be provided to the participant. If applicable, it will be provided in a certified translation of the local language. All signed and dated ICFs must remain in each participant's study file and must be available for verification by study monitors at any time.

The ICF should be revised whenever there are changes to procedures outlined in the informed consent or when added information becomes available that may affect the willingness of the participant to participate. For any updated or revised ICFs, the medical file for each participant should document the informed consent process and that written informed consent was obtained for the updated/revised ICF for continued participation in the study.

9.4 Participant Confidentiality

To maintain participant confidentiality, each participant will be assigned a unique participant identifier upon study enrolment. This participant identifier will be used in place of participant name for data analysis and reporting. Medical record numbers or other local reference identifiers are not collected as part of the database. All parties will ensure the protection of participant personal data and will not include participant names on any study forms, reports, publications, or in any other disclosures, except where required by law. In accordance with local regulations in each of the registry countries, participants will be informed about data handling procedures and asked for their consent. Data protection and privacy regulations will be observed in capturing, forwarding, processing, and storing participant data. Every effort will be made to protect participant confidentiality in compliance with the Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons about the processing of personal data and on the free movement of such data and repealing Directive 95/46/EC (General Data Protection Regulation).

The database will be housed at IQVIA in a physically and logically secure computer system kept by IQVIA as per a written security policy. The system meets approved, established standards for the security of health information and is validated. The system also meets the standards of the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use Good Clinical Practice E6 guideline (revision 2) regarding electronic study data handling and is available for audit upon request.

9.5 Independent Ethics Committee/Institutional Review Board

Consistent with local regulations and prior to enrolment of participants at a given site, the study protocol will be submitted together with its associated documents (e.g., ICF, information sheet) to the IRB/IEC responsible for its review. Participant enrolment will not start at any site before the Sponsor has obtained written confirmation of a favourable opinion/approval from the

relevant central or local IRB/IEC. The IRB/IEC will be asked to provide documentation of the date of the meeting at which the favourable opinion/approval was given that clearly identifies the study, the protocol version, and the ICF version reviewed.

Before implementation of any substantial changes to the protocol, protocol amendments will also be submitted to the relevant IRB/IEC in a manner consistent with local regulations. Pertinent safety information will be submitted to the relevant IECs during the study in accordance with local regulations and requirements. It is the responsibility of the investigator to have prospective approval of the study protocol, protocol amendments, and ICF, and other relevant documents, if applicable, from their local IRB/IEC and provide documentation of approval to the Sponsor or IQVIA. All correspondence with the IRB/IEC should be retained in the investigator file.

Should the study be terminated early for any unanticipated reason, the investigator will be responsible for informing the IRB/IEC of the early termination.

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Myositis Functional Index-2 (FI-2), Myositis Activities Profile (MAP), Inclusion Body Myositis Functional Rating Scale (IBMFRS), Cutaneous Dermatomyositis Disease Area and Severity Index (CDASI), Cutaneous Assessment Tool (CAT), Dermatomyositis Skin Severity Index (DSSI), Skindex, and Dermatology Life Quality Index (DLQI). Arthritis Care Res (Hoboken). 2011;63 Suppl 11(0 11):S118-S157.

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11. APPENDIX

11.1 Full physical, neurological, and functional examination

The following tests will be conducted as part of the physical, neurological, and functional examination:

- MMT of key muscle movements (scored according to the MRC scale), including:
    - Neck flexor and extensor (note that neck flexion will be assessed separately as part of the tests conducted for the primary objective endpoints);
    - Deltoideus;
    - Biceps;
    - Triceps;
    - Wrist flexor and extensor;
    - Finger flexor, extensor, and spreader;
    - Iliopsoas;
    - Gluteus maximus;
    - Quadriceps;
    - Hamstrings;
    - Tibialis anterior; and
    - Gastrocnemius.
- Walking gait (Normal or Abnormal);
- Walking on heels (Yes or No);
- Walking on toes (Yes or No);
- Reflexes of biceps, triceps, knees, and achilles (scored as: 0 = No contraction; 1 = Decreased, but still present; 2 = Normal);
- Sensation of upper and lower extremities (Normal or Abnormal);
- High arched palate (Yes or No);
- Orthopaedic/dysmorphic features;
- Eye movements;
- Ptosis (Yes or No);
- Facial weakness (Yes or No); if Yes, further assess for:
    - Strength of eye closure;

- Ability to whistle (Yes or No); and
- Ability to puff out cheeks (Yes or No);

- Joint hypermobility, scored using the Beighton scale (50).

Further details about the scoring and derived variables for each test conducted during the physical, neurological, and functional examination will be outlined in the SAP.

11.2 PROMIS Fatigue Short Form v1.0- Fatigue 7a (41, 42)

PROMIS® Item Bank v.1.0 - Fatigue -Short Form 7a

Fatigue - Short Form 7a

Please respond to each question by marking one box per row.

In the past 7 days…

		Never	Rarely	Sometimes	Often	Always
FATEXP20	How often did you feel tired?.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
FATEXP6	How often did you experience extreme exhaustion?.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
FATEXP18	How often did you run out of energy?.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
FATIMP33	How often did your fatigue limit you at work (include work at home)?.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
FATIMP30	How often were you too tired to think clearly?.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
FATIMP21	How often were you too tired to take a bath or shower?.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
FATIMP40	How often did you have enough energy to exercise strenuously?.....	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

11.3 PROMIS Physical Function Short Form v2.0 10a (43)

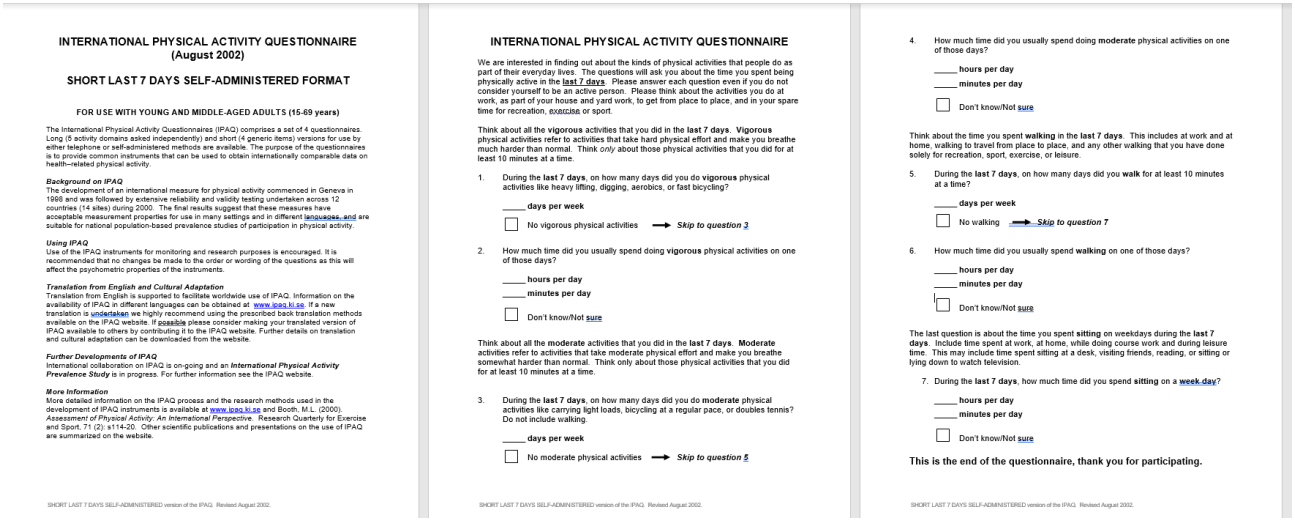
PROMIS® Item Bank v2.0 – Physical Function – Short Form 10a

Physical Function – Short Form 10a

Please respond to each question or statement by marking one box per row.

		Not at all	Very little	Somewhat	Quite a lot	Cannot do
PFA1	Does your health now limit you in doing vigorous activities, such as running, lifting heavy objects, participating in strenuous sports?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
PFC36r1	Does your health now limit you in walking more than a mile (1.6 km)?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
PFC37	Does your health now limit you in climbing one flight of stairs?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
PFA5	Does your health now limit you in lifting or carrying groceries?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
PFA3	Does your health now limit you in bending, kneeling, or stooping?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
		Without any difficulty	**With a little difficulty**	**With some difficulty**	**With much difficulty**	**Unable to do**
PFA11	Are you able to do chores such as vacuuming or yard work?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
PFA16r1	Are you able to dress yourself, including tying shoelaces and buttoning your clothes?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
PFB26	Are you able to shampoo your hair?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
PFA55	Are you able to wash and dry your body?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
PFC45r1	Are you able to sit on and get up from the toilet?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

11.4 International Physical Activity Questionnaire (IPAQ) Short Form (46)



INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE
(August 2002)

SHORT LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

Background on IPAQ
The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ
Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation
Translation from English is supported to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ
International collaboration on IPAQ is on-going and an ***International Physical Activity Prevalence Study*** is in progress. For further information see the IPAQ website.

More Information
More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective*. Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

SHORT LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised August 2002.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

   _____ **days per week**

   ☐ No vigorous physical activities → ***Skip to question 3***

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

   _____ **hours per day**
   _____ **minutes per day**

   ☐ Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

   _____ **days per week**

   ☐ No moderate physical activities → ***Skip to question 5***

SHORT LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised August 2002.

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

   _____ **hours per day**
   _____ **minutes per day**

   ☐ Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

   _____ **days per week**

   ☐ No walking → ***Skip to question 7***

6. How much time did you usually spend **walking** on one of those days?

   _____ **hours per day**
   _____ **minutes per day**

   ☐ Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a week day?

   _____ **hours per day**
   _____ **minutes per day**

   ☐ Don't know/Not sure

This is the end of the questionnaire, thank you for participating.

SHORT LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised August 2002.